

Claims

1. An osteogenic device for implantation in a mammal, said device comprising:

a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and

a protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains, each of which has an amino acid sequence sufficiently duplicative of the sequence of COP-5 or COP-7 such that said protein is capable of inducing endochondral bone formation in association with said matrix when implanted in a mammal.

2. A device for implantation in a mammal, said device comprising:

a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and

a protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains, each of which has less than about 200 amino acids, in a sequence sufficiently duplicative of the sequence of

COP-5 or COP-7 such that said protein is capable of inducing cartilage formation in association with said matrix when implanted in a mammal.

3. The device of claim 1 or 2 wherein the sequence comprises:

10	20	30	40	50
CXXXXLXVXF	DXGWXXWXXP	GXXAXYCXGX	CXXPXXXXXXN	HAXX
60	70	80	90	100
QXXVXXNXXXXP	XXCCXPXXXXXXL	XXXXXXV	XLXXYXXM	VXXCXCX

wherein each X independently represents an amino acid.

4. The device of claim 1 or 2 wherein the sequence comprises:

10	20	30	40	50
LXVXF	DXGWXXWXXP	GXXAXYCXGX	CXXPXXXXXXN	HAXX
60	70	80	90	100
QXXVXXNXXXXP	XXCCXPXXXXXXL	XXXXXXV	XLXXYXXM	VXXCXCX

wherein each X independently represents an amino acid.

5. The device of claim 1 or 2 wherein the sequence comprises:

10	20	30	40	50
CKRHPLYV	DFRDVGW	NDWIVAPP	GYHAFYCH	GEC
RRRS	K S S L	QE VIS E	FD Y	E A AY
	KE F E K I	DN	L	N S Q
Q	A S	K		ITK F P
60	70	80	90	100
QTLVNSV	NPGKIP	KACCVPT	ELSAISMLY	LDENENV
SI HAI	SEQV EP	A EQMNSLAI	FFNDQDK	I RK EE T
RF	T S	K DPV V	Y N S	H RN
N S			K	RS P E

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

6. The device of claim 1 or 2 wherein the sequence comprises:

10	20	30	40	50
LYVDFRDVGWNDWIVAPPGYHAFYCHGECFPPLADHLNSTNHAI				
K S S L	QE VIS E FD Y	E A AY MPESMKAS		VI
F E K I	DN L	N S Q	ITK F P	TL
A S K				
60	70	80	90	100
QTLVNSVNPKGKIPKACCVPTELSAISMLYDENENVLKNYQDMVVECGC				
SI HAI SEQV EP	A EQMNSLAI FFNDQDK	I RK EE T DA H H		
RF T S	K DPV V	Y N S	H RN	RS
N S			K P	E

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

7. The device of claim 1 or 2 wherein the sequence comprises:

1	10	20	30	40
Vgl	CKKRHLYVEFK-DVGWQNWIAPQGYMANCYGECPYPLTE			
	50	60	70	
	ILNGSN--H-AILQTLVHSIEPED-IPLPCCVPTKMS			
	80	90	100	
	ISMLFYDNNNDNVVLRHYENMAVDECGC			

8. The device of claim 1 or 2 wherein the sequence comprises:

1	10	20	30	40
DPP	CRRHSLYVDFS-DVGWDDWIVAPLGYDAYYCHGKCPFPLAD			
	50	60	70	
	HFNSTN--H-AVVQTLVNNNNPGK-VPKACCVPTQLDS			
	80	90	100	
	VAMLYLNDQSTVVLKNYQEMTVVVGCGC			

9. The device of claim 1 or 2 wherein the sequence comprises:

1	10	20	30	40
OP1	LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS			
	50	60	70	
	YMNATN--H-AIVQTLVHFINPET-VPKPCCAPQLNA			
	80	90	100	
	ISVLYFDDSSNVILKKYRNMVVRACGCH			

10. The device of claim 1 or 2 wherein the sequence comprises:

-5  
HQRQA

1	10	20	30	40
OPL	CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS			
	50	60	70	
	YMNATN--H-AIVQTLVHFINPET-VPKPCCAPQLNA			
	80	90	100	
	ISVLYFDDSSNVILKKYRNMVVRACGCH			

11. The device of claim 1 or 2 wherein the sequence comprises:

1	10	20	30	40
CBMP-2a	CKRHPLYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD			
	50	60	70	
	HLNSTN--H-AIVQTLVNSVNS-K-IPKACCVPTELSA			
	80	90	100	
	ISMLYLDENEKVLKNYQDMVVEGGCR			

12. The device of claim 1 or 2 wherein the sequence comprises:

1	10	20	30	40
CBMP-2b	CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGDCPFPLAD			
	50	60	70	
	HLNSTN--H-AIVQTLVNSVNS-S-IPKACCVPTELSA			
	80	90	100	
	ISMLYLDEYDKVVLKNYQEMVVEGGCR			

13. The device of claim 1 or 2 wherein the sequence comprises:

1	10	20	30	40
CBMP-3	CARRYLKVDFA-DIGWSEWIISPKSFDAYYCSGACQFPMPK			
	50	60	70	
	SLKPSN--H-ATIQSIVRAVGVVPGIPEPCCVPEKMSS			
	80	90	100	
	LSILFFDENKNVVLKVYPNMTVESACR			

14. The device of claim 1 or 2 wherein the sequence comprises:

COP1 1 10 20 30 40  
LYVDFQRDVWDDWIIAPVDFDAYYCSGACQFPSAD  
50 60 70  
HFNSTN--H-AVVQTLVNNMNPKG-VPKPCCVPTELSA  
80 90 100  
ISMLYLDENSTVVLKNYQEMTVVGCGR

15. The device of claim 1 or 2 wherein the sequence comprises:

COP3 1 10 20 30 40  
LYVDFQRDVWDDWIVAPPGYQAFYCSGACQFPSAD  
50 60 70  
HFNSTN--H-AVVQTLVNNMNPKG-VPKPCCVPTELSA  
80 90 100  
ISMLYLDENEKVVVLKNYQEMVVEGCGCR

16. The device of claim 1 or 2 wherein the sequence comprises:

COP4 1 10 20 30 40  
LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD  
50 60 70  
HFNSTN--H-AVVQTLVNNMNPKG-VPKPCCVPTELSA  
80 90 100  
ISMLYLDENEKVVVLKNYQEMVVEGCGCR

17. The device of claim 1 or 2 wherein the sequence comprises:

COP5 1 10 20 30 40  
LYVDFS-DVGWDDWIVAPPGYQAFYCHGECPPFLAD  
50 60 70  
HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA  
80 90 100  
ISMLYLDENEKVVVLKNYQEMVVEGCGCR

18. The device of claim 1 or 2 wherein the sequence comprises:

COP7 1 10 20 30 40  
LYVDFS-DVGWNDWIVAPPGYHAFYCHGECPPFLAD  
50 60 70  
HLNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA  
80 90 100  
ISMLYLDENEKVVVLKNYQEMVVEGCGCR

19. The device of claim 1 or 2 wherein the sequence comprises:

10  
PKHHSQRARKKNKN  
1 10 20 30 40  
COP16 CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGECPFPLAD  
50 60 70  
HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA  
80 90 100  
ISMLYLDENEKVLKNYQEMVVEGCGCR

20. The device of claim 1 or 2 wherein the osteogenics protein comprises a pair of separate polypeptide chains.

21. Osteogenic protein, produced by expression of recombinant DNA in a host cell, capable of inducing endochondral bone formation in association with a matrix when implanted in a mammal.

22. A protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains less than about 200 amino acids long in a sequence sufficiently duplicative of the sequence of COP-5 or COP-7 such that said protein is capable of inducing cartilage formation in association with a matrix when implanted in a mammal.

23. The osteogenic protein of claim 21 having an apparent molecular weight of about 30 kD when oxidized as determined by comparison to molecular weight standards in SDS-polyacrylamide gel.

24. The osteogenic protein of claim 23 further characterized by being glycosylated.

25. The osteogenic protein of claim 21 having an apparent molecular weight of about 27 kD as determined by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

26. The protein of claim 22 or 25 further characterized by being unglycosylated.

27. The protein of claim 21 or 22 comprising a pair of separate polypeptide chains.

28. The protein of claim 21 or 22 comprising the amino acid sequences:

10	20	30	40	50						
CXXX	LXV	FXDX	GWXX	XXXP	XGXX	AXY	CXG	CXXP	XXXXXXXXXX	NHAXX
60	70	80	90	100						
QXX	VXXX	NXXXX	PXXX	CCXP	XXXXXX	LXXXXXX	VXLXXX	YXXM	VXXC	CX

wherein each X independently represents an amino acid.

29. The protein of claim 21 or 22 comprising the amino acid sequences:

10	20	30	40	50						
LXV	FXDX	GWXX	XXXP	XGXX	AXY	CXG	CXXP	XXXXXXXXXX	NHAXX	
60	70	80	90	100						
QXX	VXXX	NXXXX	PXXX	CCXP	XXXXXX	LXXXXXX	VXLXXX	YXXM	VXXC	CX

wherein each X independently represents an amino acid.

30. The protein of claim 21 or 22 comprising the amino acid sequences:

10	20	30	40	50
CKRHPLYVDFRDVGWNDWIVAPPGYHAFYCHGECPPFLADHLNSTNHAIV				
RRRS K S S L	QE VIS E FD Y	E A AY MPESMKAS		VI
KE F E K I	DN	L	N S	Q ITK F P
Q A S K				TL
60	70	80	90	100
QTLVNSVNPGKIPKACCVPTELSAISMLYLDENENVVLKNYQDMVVEGGCR				
SI HAI SEQV EP A EQMNSLAI FFNDQDK I RK EE T DA H H				
RF T S	K DPV V	Y N S	H RN	RS
N S		K	P	E

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

31. The protein of claim 21 or 22 comprising the amino acid sequences:

10	20	30	40	50
LYVDFRDVGWNDWIVAPPGYHAFYCHGECPPFLADHLNSTNHAIV				
K S S L	QE VIS E FD Y	E A AY MPESMKAS		VI
F E K I	DN	L	N S	Q ITK F P
A S K				TL
60	70	80	90	100
QTLVNSVNPGKIPKACCVPTELSAISMLYLDENENVVLKNYQDMVVEGGCR				
SI HAI SEQV EP A EQMNSLAI FFNDQDK I RK EE T DA H H				
RF T S	K DPV V	Y N S	H RN	RS
N S		K	P	E

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

32. The protein of claim 21 or 22 comprising the amino acid sequences:

Vgl	1	10	20	30	40
	CKKRHLYVEFK-DVGWQNWIAPQGYMANCYGECPYPLTE				
	50	60	70		
	ILNGSN--H-AILQTLVHSIEPED-IPLPCCVPTKMSP				
	80	90	100		
	ISMLFYDNNNDNVVLRHYENMAVDECGCR				

33. The protein of claim 21 or 22 comprising the amino acid sequences:

1 10 20 30 40  
DPP CRRHSLYVDFS-DVGWDDWIVAPLGYDAYYCHGKCPFPLAD  
50 60 70  
HFNSTN--H-AVVQTLVNNNNPGK-VPKACCVPTQLDS  
80 90 100  
VAMLYLNDQSTVVLKNYQEMTVVGCGR

34. The protein of claim 21 or 22 comprising the amino acid sequence:

1 10 20 30 40  
OP1 LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS  
50 60 70  
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA  
80 90 100  
ISVLYFDDSSNVILKKYRNRMVVRACGCH

35. The protein of claim 21 or 22 comprising the amino acid sequences:

1 10 20 30 40  
OP1 CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS  
50 60 70  
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA  
80 90 100  
ISVLYFDDSSNVILKKYRNRMVVRACGCH

-5  
HQRQA

36. The protein of claim 21 or 22 comprising the amino acid sequences:

1 10 20 30 40  
CMP-2a CKRHPLYVDFS-DVGWNDWIVAPPGYHAFYCHGECFPPLAD  
50 60 70  
HLNSTN--H-AIVQTLVNSVNS-K-IPKACCVPTELSA  
80 90 100  
ISMLYLDENEKVVLKNYQDMVVEGCGCR

37. The protein of claim 21 or 22 comprising the amino acid sequences:

CBMP-2b 1 10 20 30 40  
CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGDCPFPLAD  
50 60 70  
HLNSTN--H-AIVQTLVNSVNS-S-IPKACCVPTELSA  
80 90 100  
ISMLYLDEYDKVVLKNYQEMVVEGCGCR

38. The protein of claim 21 or 22 comprising the amino acid sequences:

CBMP-3 1 10 20 30 40  
CARRYLKVDFA-DIGWSEWIISPKSFDAYYCSGACQFPMPK  
50 60 70  
SLKPSN--H-ATIQSIVRAVGVVPGIPEPCCVPEKMSS  
80 90 100  
LSILFFDENKNVVLKVVYPNMTVESACR

39. The protein of claim 21 or 22 comprising the amino acid sequences:

COP1 1 10 20 30 40  
LYVDFQRDVGVDDWIIAPVDFDAYYCSGACQFPSAD  
50 60 70  
HFNSTN--H-AVVQTLVNNNMNPGK-VPKPCCVPTELSA  
80 90 100  
ISMLYLDENSTVVLKNYQEMTVVGCGCR

40. The protein of claim 21 or 22 comprising the amino acid sequences:

COP3 1 10 20 30 40  
LYVDFQRDVGVDDWIVAPPGYQAFYCSGACQFPSAD  
50 60 70  
HFNSTN--H-AVVQTLVNNNMNPGK-VPKPCCVPTELSA  
80 90 100  
ISMLYLDENEKVVLKNYQEMVVEGCGCR

41. The protein of claim 21 or 22 comprising the amino acid sequences:

COP4 1 10 20 30 40  
LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD  
50 60 70  
HFNSTN--H-AVVQTLVNNNMNPGK-VPKPCCVPTELSA  
80 90 100  
ISMLYLDENEKVVLKNYQEMVVEGCGCR

42. The protein of claim 21 or 22 comprising the amino acid sequences:

COP5        1        10        20        30        40  
                  LYVDFS-DVGWDDWIVAPPGYQAFYCHGECPFPLAD  
                  50        60        70  
                  HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA  
                  80        90        100  
                  ISMLYLDENEKVVLKNYQEMVVEGCGCR

43. The protein of claim 21 or 22 comprising the amino acid sequences:

COP7        1        10        20        30        40  
                  LYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD  
                  50        60        70  
                  HLNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA  
                  80        90        100  
                  ISMLYLDENEKVVLKNYQEMVVEGCGCR

44. The protein of claim 21 or 22 comprising the amino acid sequences:

COP16       1        10        20        30        40  
                  CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGECPFPLAD  
                  50        60        70  
                  -10  
                  PKHHSSRARKKNKN  
                  HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA  
                  80        90        100  
                  ISMLYLDENEKVVLKNYQEMVVEGCGCR

45. The protein of claim 21 or 22 comprising the product of expression of a DNA in a procaryotic cell.

46. A DNA sequence encoding an amino acid sequence sufficiently duplicative of that of the sequence encoded by the gene of Figure 1A\_ such that said encoded sequence induces bone or cartilage formation when implanted in a mammal in association with a matrix.

47. The DNA of claim 46 encoding the same amino acid sequence as the gene set forth in Figure 1A.

48. The DNA sequence of claim 46 encoding:

OP1 1 10 20 30 40  
LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS  
50 60 70  
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA  
80 90 100  
ISVLYFDDSSNVILKKYRNMVVACGCH

49. The DNA sequence of claim 46 encoding:

OP1 1 10 20 30 40  
-5  
HQRQA  
CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS  
50 60 70  
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA  
80 90 100  
ISVLYFDDSSNVILKKYRNMVVACGCH

50. A cell line engineered to express the protein of claim 21 or 22.

51. The protein of claim 21 having a half maximum bone forming activity of about 20 - 25 ng per 25 mg of implant.

52. A biocompatible, in vivo biodegradable deglycosylated collagenous matrix defining pores of dimensions sufficient to permit influx, proliferation, and differentiation of migratory progenitor cells from the body of a mammal.

53. The matrix of claim 52 comprising close-packed particulate matter having a particle size within the range of 70-850 nm.

54. The matrix of claim 53 wherein said particulate matter has a particle size within the range of 70-420 mm.

55. The matrix of claim 52 defining a shape to span a non-union fracture in said mammal.

56. The matrix of claim 52 comprising demineralized, protein-extracted, deglycosylated, particulate xenogenic bone.

57. The matrix of claim 52 comprising a material selected from the group consisting of hydroxyapatite, tricalcium phosphate, polymers comprising lactic acid monomer units, polymers comprising glycolic acid monomer units, demineralized, guanidine-extracted, deglycosylated xenogenic bone, and mixtures thereof.

58. An osteogenic device for implantation in a mammal, said device comprising:

a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and

substantially pure osteogenic protein capable of inducing endochondral bone formation in said mammal disposed in said matrix and accessible to said cells.

59. The device of claim 1, 2, or 58 wherein said matrix comprises close-packed particulate matter having a particle size within the range of 70-850 mm.

60. The device of claim 1, 2, or 58 wherein said particulate matter has a particle size within the range of 70-420 mm.

61. The device of claim 1, 2, or 58 wherein said matrix comprises demineralized, protein-extracted, particulate, allogenic bone.

62. The device of claim 1, 2, or 58 wherein said matrix comprises a material selected from the group consisting of collagen, hydroxyapatite, tricalcium phosphate, polymers comprising lactic acid monomer units, polymers comprising glycolic acid monomer units, demineralized, guanidine-extracted allogenic bone, and mixtures thereof.

63. The device of claim 1, 2, or 58 wherein said matrix is shaped to span a non-union fracture in said mammal.

64. The device of claim 1, 2, or 58 disposed within the marrow cavity of allogenic bone.

65. The device of claim 1, 2, or 58 wherein said matrix comprises demineralized, protein extracted, particulate, deglycosylated xenogeneic bone.

66. The device of claim 65 wherein said matrix is treated with a protease.

67. The device of claim 58 wherein said osteogenic protein is unglycosylated.

68. The device of claim 67 wherein said osteogenic protein has an apparent molecular weight of about 27 kD when oxidized as determined by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

69. The device of claim 58 wherein said osteogenic protein is glycosylated.

70. The device of claim 69 wherein said osteogenic protein has an apparent molecular weight of about 30 kD when oxidized as determined by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

71. The device of claim 58 wherein said osteogenic protein comprises a pair of polypeptide chains.

72. The device of claim 71 wherein one chain of said pair of polypeptide chains has an apparent molecular weight of about 14 kD and the other has an apparent molecular weight of about 16 kD, both as determined after reduction by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

73. The device of claim 71 wherein one chain of said pair of polypeptide chains has an apparent molecular weight of about 16 kD and the other has an apparent molecular weight of about 18 kD, both as determined after reduction by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

74. The device of claim 58 wherein said osteogenic protein has the approximate amino acid composition set forth below:

<u>Amino acid residue</u>	<u>Rel. no. res./molec.</u>	<u>Amino acid residue</u>	<u>Rel. no. res./molec.</u>
Aspartic acid/	22	Tyrosine	11
Asparagine		Valine	14
Glutamic acid/	24	Methionine	3
Glutamine		Cysteine	16
Serine	24	Isoleucine	15
Glycine	29	Leucine	15
Histidine	5	Proline	14
Arginine	13	Phenylalanine	7
Threonine	11	Tryptophan	ND
Alanine	18		
Lysine	12		

75. The device of claim 58 wherein said osteogenic protein comprises the amino acid sequence:

VPKPCCAPT

76. The device of claim 1 or 58 wherein the half maximum bone inducing activity of said protein is 0.8 to 1.0 ng per mg of said matrix.

77. A method of inducing local cartilage or bone formation in a mammal comprising the step of implanting the device of claim 1, 2, or 58 in said mammal at a locus accessible to migratory progenitor cells of said mammal.

78. A method of inducing endochondral bone formation in a mammal comprising the step of implanting the device of claim 1 or 58 in said mammal at a locus accessible to migratory progenitor cells of said mammal.

79. A method of inducing endochondral bone formation in a non-union fracture in a mammal comprising the step of implanting in the fracture in said mammal the device of claim 63.

80. Antibodies reactive with an epitope of the protein of claim 21 or 22.